YAP1-fusions in pediatric NF2-wildtype meningioma

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Meningioma is the most common primary central nervous system (CNS) tumor [8]. In contrast to adulthood, meningiomas are rare among children and adolescents and frequently (about 38%) occur in the context of tumor predisposition syndromes [12]. In line with the frequent inactivation of *NF2* in adult meningiomas, neurofibromatosis type 2 is the most common inherited syndrome predisposing to the early development of meningiomas, which are often multiple. Other germline alterations predisposing to meningioma development are *SMARCE1* [14] and *SUFU* mutations [1]. More recently identified drivers of meningiomas include *AKT1*/*TRAF7*, *SMO*, *KLF4*/*TRAF7*, and *PIK3CA* mutations [3, 5].

The mutational underpinnings of sporadic pediatric meningioma have remained elusive to date. We report in-frame gene rearrangements predicted to result in fusions involving *YAP1* in nine meningiomas. We initially identified a *YAP1-MAML2* fusion by clinical RNA sequencing in a four year old female patient with an intraventricular mass, histologically compatible with meningioma. Subsequently, based on our database of >30,000 DNA methylation profiles of brain tumors [4], including >1,000 meningiomas (among them about 102 pediatric meningiomas, defined as age of diagnosis equal or below 18 years) [13], and corresponding copy number information, we additionally identified eight meningiomas with structural alterations affecting chromosome 11q around the *YAP1* locus (Online Resources Supplementary Fig. 2 and 3). All clustered with reference meningioma cases in t-SNE analysis of DNA methylation data (Fig. 1a) and showed histological and immunohistochemical features of meningioma (Online Resource Supplementary Fig. 1), despite two being initially diagnosed as glioma. Interestingly, of the nine cases two were classified as transitional and two as atypical meningiomas - both subtypes which often carry *NF2* mutations. With the presented case number and incomplete information on few samples, robust conclusions are, however, not derivable on subtype distribution. This limitation also applies to localizations. The clinicopathological characteristics are summarized in Table 1. RNA (seven samples) or exome (one sample) sequencing [15] revealed the presence of *YAP1* fusions in all eight additional tumors (Fig. 1b-d and Online Resource Supplementary Table 1).

Seven tumors harbored a rearrangement of *YAP1-MAML2* involving exons 1-5 (n=5) or only in exon 1 (n=2) of *YAP1* (NM_001130145) and exon 2-5 of *MAML2* (NM_032427). *YAP1-MAML2* fusions were verified by fluorescence *in situ* hybridization (FISH) performed in two cases (Online Resource Supplementary Fig. 4). A *YAP1-PYGO1* fusion was seen in a single case, containing exons 1-4 of *YAP1* and exons 2-3 of *PYGO1* (NM_015617). Additionally, a *YAP1-LMO1* fusion was detected in another case involving exons 1-4 of *YAP1* and exons 2-4 of *LMO1*. Of note, seven of the additional eight patients were children or adolescents, whereas one patient was an adult.

YAP1 is a transcriptional co-activator and downstream effector of the HIPPO pathway that acts mainly through TEAD family transcription factors and regulates expression of genes involved in cell proliferation and apoptosis [6, 7, 19, 20]. Deregulation of the HIPPO pathway via overexpression of *YAP1*, leading to tumorigenesis is a frequent event in human malignancies including meningiomas [2, 9]. Rearrangements involving the *YAP1* gene have also recently been implicated as a driver in different types of cancer. Valouev et al. reported an in-frame gene fusion between *YAP1* and *MAML2* in nasopharyngeal carcinomas [16]. A similar fusion between *YAP1* and *MAMLD1* has been described in ependymoma [10, 11]. Both *MAML2* and *MAMLD1* are members of the Mastermind gene family and act as transcriptional co-activators of NOTCH signaling [17]. The *YAP1-MAML2* rearrangement combines the transcriptional activation domain of *MAML2* with the TEAD-binding domain of

YAP1, which likely results in NOTCH-independent co-activation of TEAD-mediated HIPPO signaling [17]. While *PYGO1* has been associated with different types of cancer, structural rearrangements including *YAP1* and *PYGO1* have not been reported to the best of our knowledge. Notably, PYGO1 also functions as a transcriptional co-activator in the Wnt pathway. LMO1 acts as a transcriptional regulator with a tumor-promoting activity, but its role in tumors has not been well studied.

These alterations seem to act as an alternative to *NF2* inactivation, since no *NF2* alterations were detected in the present cohort. The *NF2* gene product, the tumor suppressor merlin, functions upstream of the HIPPO pathway and there is growing evidence suggesting a functional link between *NF2*, *YAP1* and activation of the HIPPO pathway [2, 18]. In line, *YAP1* fusion positive meningiomas clustered closer to *NF2* mutant cases than other pediatric meningiomas (Online Resource Supplementary Fig. 5). This observation parallels the virtually mutually exclusive alterations of *NF2* and *YAP1* in ependymoma. Since DNA methylation correlates with cell-of-origin, the proximity of *YAP1*- and *NF2*-altered cases in the clustering might indicate more similarities of their precursor cells compared to *SMARCE1* mutant meningiomas. However, the lack of *AKT1*, *SMO*, or *KLF4/TRAF7* mutant pediatric meningiomas and the few *SMARCE1* cases are limitations of this analysis.

Our findings identify *YAP1* fusions as a potential oncogenic driver in the development of meningiomas, predominantly in pediatric patients, and strengthen the hypothesis that deregulation of the HIPPO pathway is a central mechanism in meningioma tumorigenesis. Further studies in larger cohorts are needed to determine additional downstream functional consequences and a possible prognostic role of *YAP1* alterations in meningiomas.

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Figure legends

Fig. 1 a) Unsupervised hierarchical clustering of DNA methylation profiles in nine *YAP1*-fused meningiomas (MNG_YAP1) alongside 128 well-characterized CNS neoplasms and control tissue shown in a two-dimensional representation of pairwise sample correlations using the 15,000 most variant probes by t-distributed stochastic neighbor embedding (t-SNE) dimensionality reduction. Reference methylation classes: ependymoma, posterior fossa group A (EPN_PFA), ependymoma, posterior fossa group B (EPN_PFB), ependymoma, RELA fusion (EPN_RELA), ependymoma, YAP fusion (EPN_YAP), subependymoma, posterior fossa (EPN_PF_SE), ependymoma, spinal (EPN_SPINE), subependymoma, spinal (EPN_SPINE_SE), subependymoma, supratentorial (EPN_ST_SE), meningioma (MNG), chordoma (CHORDM), melanoma (MELN), schwannoma (SCHW) and control tissue white matter (CONTROL). Schematic of the *YAP1-MAML2* fusion involving exons 1-5 of *YAP1* and exons 2-5 of *MAML* **(b)**, the *YAP1-PYGO1* fusion involving exons 1-4 of *YAP1* and exons 2-3 of *MAML2* **(c)** and the *YAP1-LMO1* fusion involving exons 1-4 of *YAP1* and exons 2-4 of *LMO1* **(d)**.

Table1 Clinicopathological characteristics of the *YAP1*-fused meningioma cohort

Case #	Age (years)	Sex	Tumor location	Initial Diagnosis	Genetic alteration
1	4	F	lateral ventricles, 3rd ventricle	MNG	*YAP1:MAML2*
2	3	M	temporal	PXA	*YAP1:PYGO1*
3	1	M	3rd ventricle, lateral ventricle	pHGG	*YAP1:MAML2*
4	2	M	skull base	MNG	*YAP1:MAML2*
5	36	M	optic nerve	MNG	*YAP1:MAML2*
6	8	F	skull base (supra-/infratentorial)	MNG	*YAP1:LMO1*
7	17	M	cavernous sinus	MNG	*YAP1:MAML2*
8	7	F	parietal	MNG	*YAP1:MAML2*
9	7	F	frontal	MNG	*YAP1:MAML2*

MNG – meningioma, PXA – pleomorphic xanthoastrocytoma, pHGG – pediatric high grade glioma, F – female, M -
subtypes meningiomas and WHO grade are provided in Supplementary Table 2.

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Supplementary materials and methods

Sample collection

Besides the index cases emerged in routine diagnostics, additional cases of *YAP1*-fused meningioma and reference cases were retrieved from the archives of the Departments of Neuropathology at the University Hospitals in Heidelberg, Magdeburg (both Germany) and Zürich (Switzerland), the Department of Pediatrics, McGill University, and The Research Institute of the McGill University Health Center, Montreal (Canada) as well as the Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN (USA). Analysis of tissue and clinical data was performed in accordance with local ethical regulations.

Immunohistochemistry

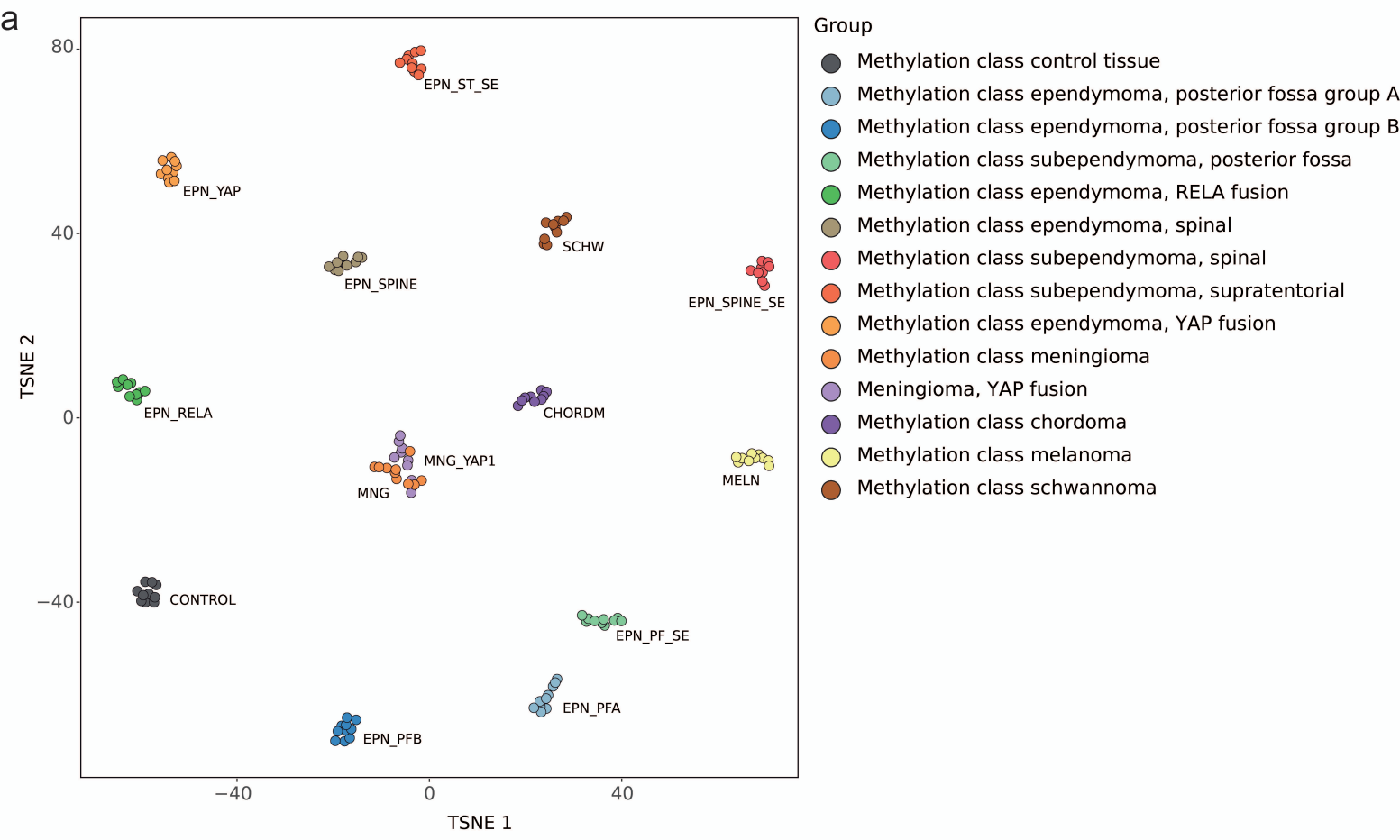
Immunohistochemistry was performed on a Ventana BenchMark ULTRA Immunostainer (Ventana Medical Systems, Tucson, AZ, USA) for all cases with sufficient material (n=5). Antibodies were directed against: epithelial membrane antigen (EMA; Clone GP1.4, mouse monoclonal, dilution 1:1000, Thermo Fisher Scientific, Fremont, CA, USA), somatostatin receptor 2A (SSTR2A; SS-8000-RM, rabbit monoclonal, dilution 1:10, Biotrend, Cologne, Germany) and Ki-67 (clone MIB-1, mouse monoclonal, 1:100 dilution, Dako Agilent, Santa Clara, CA, USA).

Nucleic acid extraction, DNA methylation analysis and RNA/exome sequencing

Representative tumor tissue with highest tumor cell content was histologically identified and selected for nucleic acid extraction using local standard protocols. DNA methylation profiling of all samples was performed using the Infinium MethylationEPIC (850k) BeadChip (Illumina, San Diego, CA, USA) or Infinium HumanMethylation450 (450k) BeadChip (Illumina) array according to the manufacturer's instructions. Filtering and genome-wide copy number analyses were performed as previously described, using the 'conumee' package in R (http://www.bioconductor.org) [2]. RNA and DNA sequencing were performed on a NextSeq 500 (Illumina) as previously described [1]. Fusion discovery used defuse and arriba (https://github.com/suhrig/arriba/).

Fluorescence *in situ* hybridization (FISH)

FISH was performed using the YAP1 Break Apart Probe (Empire Genomics, Williamsville, NY, USA) on interphase nuclei on 5µm FFPE sections in two cases according to the standard procedures and manufacturer's instructions. The 5' centromeric and 3' telomeric probes are labeled with 5-carboxyl-x-rhodamine and 5-fluorescein, respectively.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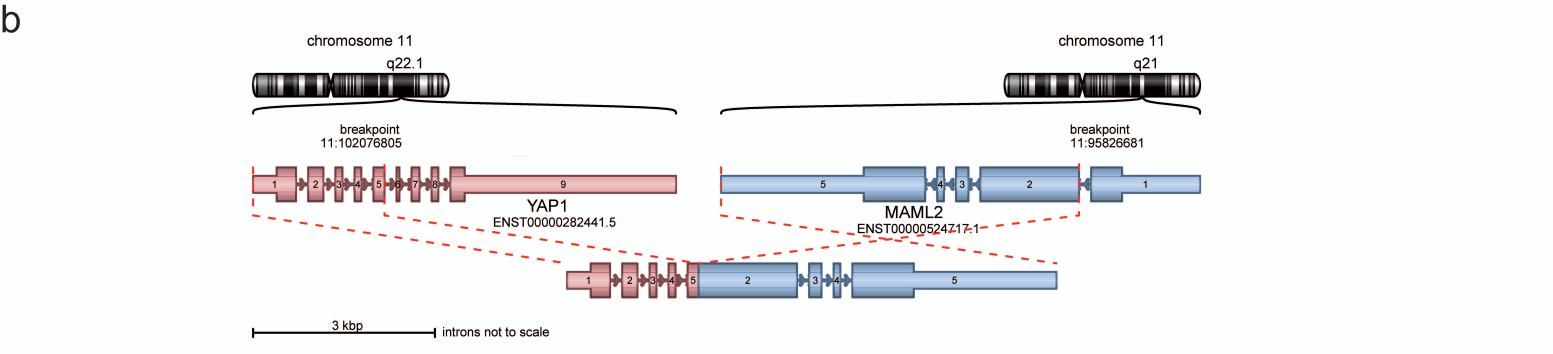
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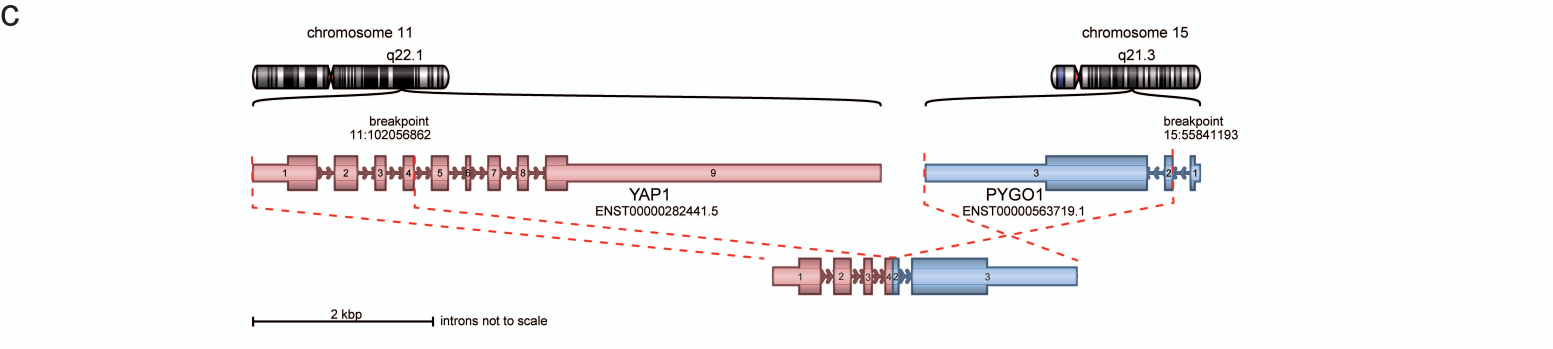
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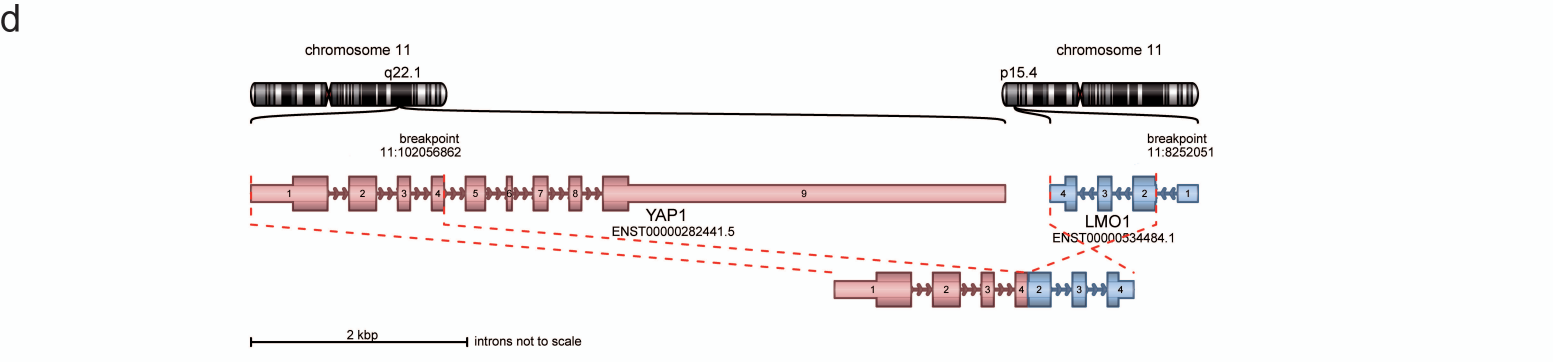
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